Indonesian Journal of Global Health Research
Volume 6 Number 5, October 2024
e-ISSN 2715-1972; p-ISSN 2714-9749
http://jurnal.globalhealthsciencegroup.com/index.php/IJGHR

CHARACTERIZATION AND ANALYSIS OF FLAVONOID IN ETHANOL EXTRACT AND PURIFIED EXTRACT OF FRINGED SPIDER FLOWER (CLEOME RUTIDOSPERMAE DC.) AS ANTIOXIDANTS

Prischilla Christyaningrum, Nastiti Utami*, Alip Desi
Undergraduate Pharmacy Study Program, Sekolah Tinggi Ilmu Kesehatan Nasional, Jl. Raya Solo - Baki, Bangorwo, Kwarasan, Sukoharjo, Central Java 57552, Indonesia
*Nastiti.utami@stikesnas.ac.id

ABSTRACT

The secondary metabolite profile of fringed spider flower or purple cleome (Cleome rutidospermae DC.) has not been widely explored. One of the secondary metabolites contained in purple cleome is flavonoids. Flavonoids have antioxidant, antibacterial, antidiabetic activities. Objective: This study aims to qualitative analysis of flavonoid, determine total flavonoid content, and antioxidant activity of ethanol extracts and purified extracts. Method: Purple cleome leaf was extracted with 96% ethanol using maceration method for 5 days, followed by extract purification stage. Qualitative identification of flavonoids using Wilstater and Taubeck methods. Identification of flavonoid compounds in the UV-Vis spectrum was based on the presence of typical cinnamoyl and benzoyl bands, determination of flavonoid content using aluminum chloride colorimetric method, and antioxidant activity test using DPPH method. Results: Qualitative identification of ethanol extract and purified extract of purple cleome have positive result contained flavonoids using the wilstater and taubeck tests. Identification of flavonoids with UV-Vis, the ethanol extract showed absorption of flavone, substituted flavonol 3-OH, and free flavonol 3-OH, while the purified extract showed absorption of flavone and free flavonol 3-OH. The ethanol extract and purified extract had total flavonoid content of 7.625±0.000 mg QE/g extract and 2.396±0.036 mg QE/g extract. Antioxidant activity results on ethanol extracts and purified extracts obtained IC50 values of 128.249±0.070 ppm and 86.513±0.042 ppm. Conclusions: The highest flavonoid content was found in the ethanol extract of purple cleome leaf. Flavonoid types in ethanol and purified extracts of purple cleome leaf indicated flavone and flavonol types based on the wavelengths of cinnamoyl and benzoyl. The strongest antioxidant category was found in the purified extract of purple cleome.

Keywords: antioxidant; cleome rutidospermae; flavonoid; purple cleome; sieruela rutidosperma (dc.) roalson & j.c.hall.

First Received	Revised	Accepted
28 March 2024	28 April 2024	30 April 2024
Final Proof Received		**Published**
07 July 2024		01 October 2024

How to cite (in APA style)
Christyaningrum, P., Utami, N., & Desi, A. (2024). Characterization and Analysis of Flavonoid in Ethanol Extract and Purified Extract of Fringed Spider Flower (Cleome Rutidospermae Dc.) as Antioxidants. Indonesian Journal of Global Health Research, 6(5), 3011-3020. https://doi.org/10.37287/ijghr.v6i5.3760.

INTRODUCTION

Traditional medicine development need to adhere to strict standards in terms of quality, safety, and efficacy. Therefore, the development of traditional medicine must be supported by scientific data, one of which is by knowing the composition of the active substances in effective medicinal ingredients. Natural medicinal ingredients contain secondary metabolite compounds which efficacious as active substances, where these substances function to help produce therapeutic effects (Pratiwi et al., 2022). In India, Ayurvedic practitioners have utilized the compounds in the leaves, roots, and seeds of the purple cleome (Cleome rutidospermae D.C.) plant have biological activities including antipyretic, diuretic, anti-inflammatory, antibacterial, antidiabetic (Okoro et al., 2014), and antioxidant (Ghosh et al.,

2019), epilepsy, paralysis, convulsions, pain, and skin diseases (Rajesh et al., 2012). It is commonly found growing in the tropical and subtropical regions of Asia and Africa. Purple cleome is known for its distinctive, spider-like flowers that bloom in shades of pink, purple, and white (Rojas et al., 2022).

Flavonoids, phenols, tannins, alkaloids, steroids, saponins, and terpenoids are found in purple cleome leaf (Ghosh et al., 2019). Flavonoid in purple cleome leaf indicates that it has antioxidant properties. Flavonoids are included in the polyphenol group that have the potential as antioxidant, anti-inflammatory, antihepatotoxic, antitumor, and antibacterial activities (Rizqianingrum, 2020). Research on chemical compounds contained in purple cleome leaf is limited, so that research on the composition of these compounds is needed to help develop purple cleome leaf as traditional medicine. The objective of this research is to determine the UV-Vis spectrum profile of flavonoid compounds, the total of flavonoid compounds, and the antioxidant potential of the ethanol extract and purified extract of purple cleome leaf. Therefore, this study was conducted as a first step to develop traditional medicine by analyzing flavonoid levels and characterizing flavonoid types in ethanol and purified extracts of purple cleome leaf as antioxidants.

METHOD

Sample collection

Purple cleome leaf used in this study were obtained from Karangdowo, Klaten, Central Java and harvested in the afternoon.

Sample preparation

Samples of purple cleome leaf was determined at the Tawangmagu Traditional Health Service Functional Implementation Unit (UPF Yankestrad Tawangmangu) by matching the morphological characteristics of purple cleome plants. Purple cleome leaf was sorted and washed, then the sample was dried for 72 hours in the oven at 50°C. The dried purple cleome leaf were reduced in size and sieved on mesh no. 40.

Extraction

500 grams purple cleome leaf powder was macerated with 96% ethanol as much as 3.75 liters for 3 days and stirred once every 24 hours. The maceration results were filtered, the residue of the maceration results was added to 1.25 liters of 96% ethanol for remaceration for 2 days. The filtrate from maceration and remaceration was combined and then concentrated to obtain a thick extract using a rotary evaporator and waterbath at 50°C.

Extract purification

Ethanol extract purple cleome (EEPC) was purified by liquid-liquid extraction method. 25 grams of EEPC was added 250 mL of hot water, then the extract solution was added 250 mL of n-hexane until two layers were formed, then the two layers were separated. The water phase was partitioned with ethyl acetate as much as 250 mL and separation is carried out between the ethyl acetate phase and water phases. The fractionation results are concentrated to obtain a thick ethyl acetate fraction referred to as purified extract of purple cleome leaf (PEPC).

Phytochemical screening of flavonoids

Wilstater test

A total of 0.1 gram of EEPC and PEPC were added with magnesium (Mg) powder and

concentrated HCl as much as 3-4 drops. The color change to red-orange in the extract indicates positive flavonoid content (Pratiwi et al., 2022).

Taubeck test

A total of 0.1 gram of EEPC and PEPC was added to distilled water and then evaporated to dryness with a waterbath, added with acetone, boric acid and oxalic acid. The mixture was evaporated on a waterbath and added ether then observed at UV light 366 nm. The presence of yellow fluorescence under UV light 366 nm indicates positive flavonoid content (Djamil and Zaidan et al., 2016).

Flavonoid Characterization with UV-Vis Spectrophotometer

EEPC and PEPC were each weighed 0.005 g then dissolved using methanol p.a in a 5 mL volumetric flask. Characterization was carried out by analyzing that solution using a UV-Vis spectrophotometer at a wavelength of 220-400 nm. The analysis results indicated the presence of the first bond (band I) in the range of 300-400 nm indicating the cinnamoyl system and the second bond (band II) 240-285 nm indicating the benzoyl system.

Quantitative Test of Flavonoid Content

Preparation of 1000 ppm standard solution

A total of 10.0 mg of quercetin standard was dissolved with ethanol p.a in a 10 mL volumetric flask.

Preparation of 100 ppm intermediate solution

The parent standard solution was pipetted as much as 1 mL added to ethanol p.a in a 10 mL volumetric flask to obtain a concentration of 100 ppm.

Preparation of blank solution

Pipetted 1 mL of 10% $AlCl_3$ solution and 5% acetic acid as much as 8 mL, and added ethanol p.a to the limit mark of a 10 mL volumetric flask.

Determination of operating time

Intermediate solution was pipetted as much as 1 mL, added $AlCl_3$ 10% as much as 1 mL and 5% acetic acid 8 mL. Measurement of operating time was carried out at the theoretical wavelength of quercetin 415 nm by using UV-Vis spectrophotometer (Ipandi *et al.*, 2016).

Determination of the maximum wavelength of quercetin

Intermediate solution was pipetted as much as 1 mL and reacted with 1 mL of 10% $AlCl_3$ and 8 mL of 5% acetic acid. Measurement of maximum wavelength was carried out at OT and wavelength of 400-450 nm by using UV-Vis spectrophotometer.

Preparation of standard curve of quercetin

The concentration of the standard series was made 50, 70, 90, 110, and 130 ppm. Each concentration of the standard series was pipetted as much as 1 mL, and added 1 mL of 10% $AlCl_3$ and 8 mL of 8% acetic acid, allowed to stand for 26 minutes. Determination of absorbance of the standard curve was measured at a wavelength of 415 nm.

Determination of total flavonoid content

EEPC and PEPC as much as 160 mg was dissolved with ethanol p.a up to 10 mL in a

volumetric flask. Each sample was pipetted 1 mL, added 10% $AlCl_3$ as much as 1 mL and 5% acetic acid 8 mL, allowed to stand for 26 minutes and then measured the absorbance of the sample using UV-Vis spectrophotometry.

Antioxidant Activity Test

Preparation of blank solution

A total of 2 mL of 0.4 mM DPPH solution was added with ethanol p.a in a 10 mL volumetric flask.

Measurement of maximum wavelength

A total of 1 ml of 0.4 mM DPPH solution was added with ethanol p.a in a 5 mL volumetric flask. Incubated ±30 minutes. Absorbance was measured at range wavelength of 400-800 nm by using UV-Vis spectrophotometry. The results obtained will obtain the maximum wavelength and absorbance value of 0.4 mM DPPH solution.

Measurement of operating time

Measurement of the operating time of the 2 ml each ascorbic acid, EEPC, and PEPC adding 2 mL of DPPH solution and measuring the absorbance at a wavelength of 516 nm by using UV-Vis spectrophotometry.

Preparation and Measurement of Ascorbic acid, EEPC, and PEPC

Concentration of 2 ppm, 4 ppm, 6 ppm, 8 ppm and 10 ppm for ascorbic acid. Concentration of 100 ppm, 110 ppm, 120 ppm, 130 ppm and 140 ppm for EEPC. Concentrations of 60 ppm, 70 ppm, 80 ppm, 90 ppm and 100 ppm for PEPC. Each concentration series was pipetted as much as 2 mL and added 2 mL of DPPH solution. The solution was incubated for 35 minutes, then the absorbance was measured at a wavelength of 516 nm by using UV-Vis spectrophotometry.

RESULTS

Phytochemical screening of flavonoids

Based on the results obtained in Table 1, it shows that EEPC and PEPC are positive for flavonoid compounds. This is supported because of the color change in the sample after adding the reagent.

Table 1.
Flavonoid phytochemical results

Sample	Test	
	Wilstater	Taubeck
Quercetin	+	+
EEPC	+	+
PEPC	+	+

Characterization of flavonoids with UV-Vis Spectrophotometer

Spectrum UV-Vis spectrophotometer could be characterization the type of flavonoids in the sample. Characterization based on the maximum absorption spectrum seen in the range of 220-400 nm.

Table 2.
Results of flavonoid spectrum

Sample	Band I (*cinnamoyl*)	Band II (*benzoyl*)	flavonoid type interpretation (Feng *et al.*, 2017)
Quercetin	372,3 nm	255,5 nm	free flavonol 3-OH
Ethanol extract (EEPC)	336,7 nm	254,6 nm 266,7 nm	flavone
	336,7 nm	254,6 nm 266,7 nm	substituted flavonol 3- OH
	369,9 nm	254,6 nm 266,7 nm	free flavonol 3-OH
Purified extract (PEPC)	323,2 nm	269,9 nm	flavone
	360,1 nm	269,9 nm	free flavonol 3-OH

The spectrum of the EEPC showed the characteristics of flavone, substituted flavonol 3- OH, and free flavonol 3-OH, while the spectrum of the PEPC showed the characteristics of flavone and free flavonol 3-OH, this explanation could be seen in Table 2 and the flavonoid spectrum of EEPC and PEPC could be seen in Figure 1.

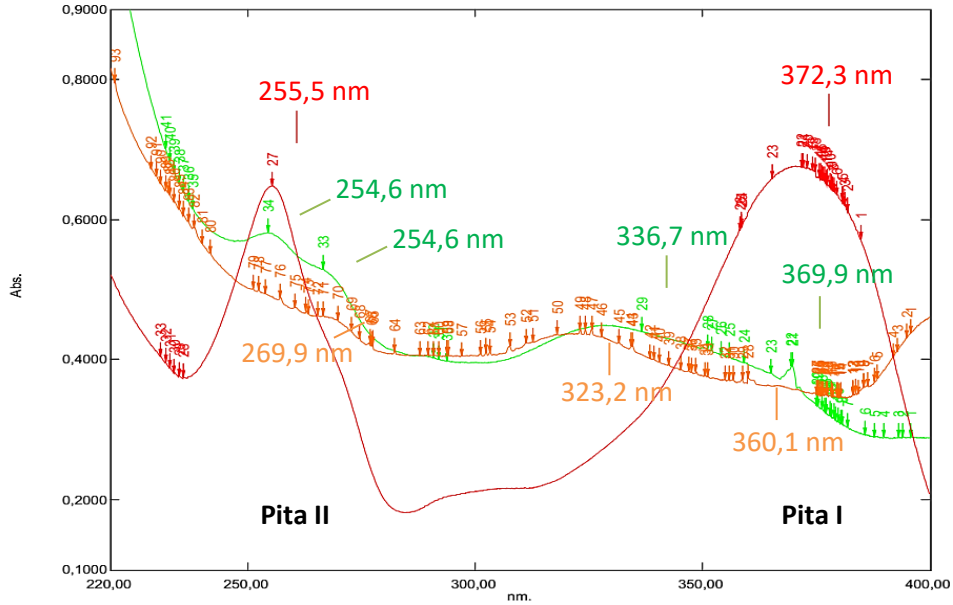

Figure 1. UV-Vis Spectra of Flavonoid Compounds

Description :
= Quercetin
= Ethanol extract (EEPC)
= Purified extract (PEPC)

Determination of total flavonoid content

Table 3 showed the two sample that have the highest flavonoid content is the EEPC.

Table 3.
The results of determining the flavonoid content of purple cleome leaves

Sample	Total Flavonoid content (mg QE/g)
EEPC	7.623±0.002
PEPC	2.396±0.036

Antioxidant activity test

The EEPC and PEPC showed free radical scavenging activity by DPPH at concentrations of 60 ppm to 140 ppm. The purified extract showed more free radical capture activity than the ethanol extract. Vitamin C as a standard also showed significant radical scavenging potential at 9 ppm concentration. The IC_{50} values of each ethanol extract. purified extract. and vitamin C can be seen in Table 4.

Table 4**.**

The Value of IC_{50} Ascorbic acid, EEPC, and PEPC

Sample	IC_{50} (ppm)
Ascorbic acid	8.920±0.062
EEPC	128.249±0.070
PEPC	86.513±0.042

DISCUSSION

Sample preparation

Determination of purple cleome leaves was carried out to ensure the plant accurately, so that there was no research sampling error. The determination results showed the species is *Cleome rutidosperma* DC. and the synonym is *Sieruela rutidosperma* (DC.) Roalson & J.C.Hall based on No. TL/02/04/D/XI.5/16536.212/2023. Purple cleome leaf powder was extracted by maceration method. The purpose of extraction is to withdraw the components of chemical compounds present in the sample by using the appropriate solvent polarity (Li & Sobańtka, 2023), 96% ethanol solvent to attract chemical compounds with a wide range of polarity. Maceration is a process of extract the sample powder without heating, so that it could protect active components that are easily damaged by heat (Istiqomah et al., 2023). Thick extracts were obtained by evaporating the filtrate using a rotary evaporator and a water bath at 50°C. Organoleptically, a thick extract with a distinctive odor and blackish green color was obtained.

Based on polarity-based chemical separation, EEPC was purified through a liquid-liquid extraction process with a separatory funnel. Immiscible solvents were used to separate compounds according to their degree of polarity, so that the compound could be dissolved in a solvent that matches its polarity. The extract was purified to prevent contaminants from ballast substance components (impurities) that could interfere with the ability of active ingredients to produce biological activity (Amalia et al., 2021). The two solvents used are ethyl acetate and n-hexane. Ethyl acetate solvent attracts semi-polar substances such as alkaloids. flavonoids. and saponins, while n-hexane solvent more effectively attracts non-polar substances such as terpenoids and sterols (Pratiwi et al., 2022).

Flavonoid phytochemical screening

Phytochemical screening of flavonoids was carried out to see the presence of flavonoid compounds in EEPC and PEPC. Quercetin is included in one of the flavonoid compounds, so that quercetin was used as a positive control in identifying flavonoid compounds. The flavonoid phytochemical results of EEPC and PEPC were positive using the Taubeck and Wilstater tests shown in Table 1. The presence of flavonoids in purple cleome in accordance with previous research (Akinsola et al., 2020). The addition of concentrated HCl makes flavonoids hydrolyzed into their aglycones by hydrolyzing O-glycosyl. Red discoloration could occur when flavonoids are reduced with magnesium powder and concentrated HCl (Rizqianingrum et al., 2020). The addition of acetone, boric acid, and oxalic acid causes the flavonoid complex to add a bathochromic shift, so that the sample fluoresces intensively yellow at UV light 366 nm (Mahanani et al., 2021).

Characterization of flavonoids with UV-Vis Spectrophotometer

The existence of flavonoid variations is due to the maximum absorption band area in each extract is different. Based on the results of data analysis in Table 2. It was found that the EEPC contains flavonoid type flavone, substituted flavonol 3-OH, and free flavonol 3-OH, while the PEPC contains flavonoid type flavone and free flavonol 3-OH. Flavonoid type flavone has a maximum absorption band area between 250-280 nm in band II and 304-350 nm in band I (Feng et al., 2017). Flavonoid type substituted flavonol 3-OH has a maximum absorption band area between 250-280 nm in band II and 328-357 nm in band I, while flavonoid type free flavonol 3-OH has a maximum absorption band area between 250-280 nm in band II and 358-385 nm in band I. Based on the analysis showed that this type of flavonoid is presence in the leaves of a plant. Flavonoids of flavone, flavonol, and flavonone types are scattered in the leaves of plants (Neldawati et al., 2013). The difference in spectrum results between sample is the compounds in EEPC more polar than PEPC such as glycosides bound to flavonoid molecules, this could be proven from the results of characterization with a UV-VIS spectrophotometer which showed the type of substituted flavonol 3-OH.

Determination of flavonoid content

Determination of total flavonoid content of purple cleome leaf was carried out using colorimetric method by forming a reaction between $AlCl_3$ with flavonoid groups to form a complex between neighboring hydroxyl and ketone groups (Shraim et al., 2021). The use of spectrophotometric methods based on the formation of aluminum complexes is one of the most commonly applied procedures for the determination of the total content of flavonoids in samples of medicinal plants (Utami et al., 2024). Determination of flavonoid content begins with the determination of operating time (OT). OT determination is done to determine the right length of time for the sample to react with the reactant to form a stable compound (Beda, 2018). OT of the reaction of quercetin was obtained at minute 26. The maximum wavelength was measured to determine the wavelength that gave the highest absorption. Measurements were carried out in the wavelength range of 400-450 nm and showed the maximum wavelength was in the 414 nm. Determination of flavonoids requires a complexing agent to increase the absorbance value and become a corrector of the extract solution which is generally light green-brown. The addition of aluminum chloride complexing agent was carried out to make a yellow solution color. The addition of acetic acid in the analysis of flavonoid content was done to form an acidic solution. This would make the C-4 keto and 3 or 5-OH stable to form a complex with $AlCl_3$ so that there is a wavelength shift towards the maximum bathochromic color (Pratiwi et al., 2022). The highest total flavonoid content in the EEPC which amounted to 7.625 ± 0.000 mg QE/g. while the lowest flavonoid content in the PEPC was 2.396 ± 0.036 mg QE/g. This difference is possible due to the flavonoid content in the ethanol extract of purple cleome leaves which is bound in the form of glycone more than the form of aglycone (Pandey et al., 2013).

Antioxidant activity test

Antioxidant activity was carried out using the DPPH method. DPPH acts as a free radical which inhibited by the antioxidants in the extract. Free radical scavenging activity was expressed by an inhibitory concentration of 50% (IC50). The ability of role of flavonoids as chain-breaking antioxidants showed in total radical scavenging activity was measured using DPPH assay (Utami & Damayanti, 2023). The maximum wavelength was measured in the range of 400-800 nm and the maximum wavelength obtained was 516 nm. The wavelength measurement aims to obtain the maximum absorption area of DPPH in order to obtain linearity results on the standard curve in the measurement of antioxidant activity. Furthermore. the determination of operating time for 0-60 minutes to determine the stable

measurement time when the sample reacts completely with the reagent. Vitamin C was used as a comparison because it has a very high antioxidant activity so that it could be used as a test of the method used is appropriate in the analysis of antioxidant activity**.**

The antioxidant value of 128.249±0.070 ppm was found in the EEPC which included in the moderate category. The PEPC has antioxidant activity with an average IC_{50} value of 86.513±0.042 ppm which included in the strong category. Research revealed that raw ethanol extract and its fractions prepared with ethyl acetate of the purple cleome aerial parts (Chakraborty et al., 2010) and leaves (Prabha et al., 2017) displayed potent antioxidant activities. Studies have reported that solvent polarity significantly affects antioxidant activity in plant material (Ghasemzadeh et al., 2015). The results obtained for total flavonoid content in the EEPC are greater than PEPC, but the antioxidant activity was stronger in PEPC. This could be influenced by the total flavonoid compounds contained in the PEPC that have an active role as antioxidants. The EEPC contain many flavonoid compounds that bind to their glycoside groups (Pandey et al., 2013), this made the antioxidant activity in EEPC lower than the PEPC because flavonoids that bind to glycoside groups have weak antioxidant activity compared to their aglycones, it cause they need time or energy to break up first with their glycosides to be able to react with radicals.

CONCLUSION

Based on the results showed that purple cleome leaves contain flavonoids of flavone type. substituted flavonol 3-OH. and free flavonol 3-OH in ethanol extracts, while in purified extracts showed flavone type and free flavonol 3-OH. The highest flavonoid content was found in the ethanol extract at 7.623± 0.002 mg QE/g followed by the purified extract at 2.396 ± 0.036 mg QE/g. The ethanol extract had moderate antioxidant activity with IC50 value of 128.249±0.070 ppm while the purified extract had strong antioxidant activity with IC50 value of 86.513±0.042 ppm.

REFERENCES

Akinsola, A.F., Oluwafemi A.G. (2022). Phytochemical analysis, proximate composition and mineral profile of Cleome rutidosperma found in Ado-Ekiti, Nigeria. International Journal of Advanced Biochemistry Research 6 (2): 94-100.

Amalia, I., Wahyuni, D., & Fikri, K. (2021). Toksisitas Ekstrak Terpurifikasi Daun Pulutan (Urena Lobata L.) Fraksi Etanol Terhadap Mortalitas Larva Nyamuk Aedes Aegypti. Saintifika, 23(2), 19-32.

Beda, T.O. (2018). Penetapan Kadar Flavonoid Total Ekstrak Etanol Daun Sisik Naga (Drymoglossum piloselloides [L.]) dengan Metode Kolorimetri AlCl3, Karya Tulis Ilmiah, Politeknik Kesehatan Kemenkes, Kupang.

Chakraborty, A.K., Charde, M.S., Roy, H., Bhanja, S., Behera, M. (2010). Comparative study of antioxidant activity between ethanolic and aqueous extract of Cleome rutidosperma. International Journal of Pharmaceutical Sciences and Research 1 (11): 112-116.

Datta, S., Bhattacharjee, S., Seal, T. (2022). Anti-diabetic, anti-inflammatory and anti-oxidant properties of four underutilized ethnomedicinal plants of West Bengal, India: an in vitro approach. South African Journal of Botany 149: 768-780.

Djamil, R., Zaidan, S. (2016). Isolasi Senyawa Flavonoid dari Ekstrak Metanol Daun Katuk (Sauropus androgynus (L.) Merr), Euphorbiaceae. Jurnal Ilmu Kefarmasian Indonesia, 14(1), 57-61

Feng, W., Zhiyou, H., Li, M. (2017). Isolation and Structure Identification of Flavonoids, INTECH.

Ghasemzadeh, A, Jaafar, H.Z., Juraimi, A.S., Tayebi-Meigooni, A. (2015). Comparative Evaluation of Different Extraction Techniques and Solvents for the Assay of Phytochemicals and Antioxidant Activity of Hashemi Rice Bran. Molecules. 20(6):10822- 10838.

Ghosh, P., Sirshendu, C., Prakriti, D., Arna, B., Srabani, K., and Supratim, M. (2019). Natural Habitat, Phytochemistry And Pharmacological Properties of A Medicinal Weed-Cleome Rutidosperma Dc. (Cleomaceae): A Comprehensive Review, IJPSR, Vol. 10(4): 1605-1612

Ghosh, P., Biswas, M., Biswas, S., Dutta, A., Hazra, L., Sudip Kumar Nag, Sayantan Sil, Sirshendu C. (2019). Phytochemical Screening, Anti-oxidant and Anti-microbial Activity of leaves of Cleome rutidosperma DC. (Cleomaceae), J. Pharm. Sci. & Res., Vol. 11(5)

Ipandi, I., Triyasmono, L., Budi, P. (2016). Penentuan Kadar Flavonoid Total dan Aktivitas Antioksidan Ekstrak Etanol Daun Kajajahi (Leucosyke capitellata Wedd.), Pharmascience, 3(1), 93-100.

Istiqomah, R., Lindawati, N.Y., Utami, N. (2023). Analisis Kadar Fenolik Total dari Ekstrak Etanol dan Ekstrak Terpurifikasi Daun Oyong (Luffa acutangula (Linn.) Roxb.), Indonesian Journal on Medical Science, Volume 10 No. 2

Larasati, R.A., Utami, N., & Lindawati, N. Y. (2023). The Effect Of Maceration Method And Soxhletation Method On Total Phenolic Content And Antioxidant Activity Of Kirinyuh (Chromolaena odorata (L.) RM King & H. Rob) LEAF. Medical Sains: Jurnal Ilmiah Kefarmasian, 8(3), 1207-1214.

Li, J., & Sobańtka, A. (2023). A Systematic Analysis of the Effect of Extraction Solvents on the Chemical Composition of Extraction Solutions and the Analytical Implications in Extractables and Leachables Studies. Journal of Pharmaceutical and Biomedical Analysis, 222, 115081. https://doi.org/10.1016/j.jpba.2022.115081

Mahanani, N., Utami, N., Pratimasari, D. (2021). Identifikasi Senyawa Flavonoid Pada Ekstrak Dan Fraksi Daun Umbi Bit (Beta vulgaris L), Journal of Pharmacy, Vol. 2, No.1.

Neldawati, Ratnawulan, Gusnedi. (2013). Analisis Nilai Absorbansi dalam Penentuan Kadar Flavonoid untuk Berbagai Jenis Daun Tanaman Obat, Pillar of Physics, Vol. 2.

Okoro, I.O.., Umar, IA., Atawodi, S.E., Anigo, KM. (2014). Antidiabetic effect of Cleome rutidosperma DC and Senecio biafrae (Oliv. & Hiern) extracts in streptozotocin-induced diabetic rats. International Journal of Pharmaceutical Sciences and Research 5 (6): 2480-2497.

Pandey, A., Kumar, S. (2013). Chemistry and Biological Activities of Flavonoids: An Overview, The Scientific World Journal.

Prabha, S.B., Rao, M., Kumar, M.R. (2017). Evaluation of in vitro Antioxidant, Antibacterial and Anticancer activities of leaf extracts of Cleome rutidosperma. Research Journal of Pharmacy and Technology 10 (8): 2492-2496.

Pratiwi, N., Utami, N., Pratimasari, D. (2022). Karakterisasi dan Penetapan Kadar Flavonoid Totak Ekstrak dan Fraksi Bunga Papaya Jantan (Carica papaya L.) dengan Spektrofotometri UV-Vis, Jurnal Ilmiah Farmasi 18 (2).

Rajesh, C.P., Swati D.W., Sunil S.Y., Vaibhay D. D. (2012). Antibacterial And Bioenhancing Activity of Ethyl Acetate Extract of Cleome rutidosperma Leaves, Journal of Pharmacy Research, 5(1).

Rizqianingrum, D., Kusnadi, Purgiyanti. (2020). Penetapan Kadar Flavonoid Total dan Uji Aktivitas Antioksidan Ekstrak Daun Seledri (Apium graveolens L). Politeknik Harapan Bersama. Tegal.

Rojas-Sandoval, J., & Acevedo-Rodríguez, P. (2022). Cleome rutidosperma (fringed spiderflower). In CABI Compendium.

Shraim, A. M., Ahmed, T. A., Rahman, M. M., & Hijji, Y. M. (2021). Determination of total flavonoid content by aluminum chloride assay: A critical evaluation. LWT, 150, 111932. https://doi.org/10.1016/j.lwt.2021.111932

Utami, N., & Damayanti, P.N. (2023). Phytochemical Analysis, Antioxidant Activity And Ftir Spectroscopic Analysis Of Red Leaf Lettuce And Green Leaf Lettuce (Lactuca Sativa L.). Indian Drugs, 60(5).

Utami, N., Damayanti, P. N., Kristy, T.K. (2024). Standarisasi dan Pemisahan Pigmen Ekstrak Etanol Daun Selada Merah (Lactuca Sativa Var Crispa L.). Jurnal Farmasetis, 13(2), 45–54.